Evidence for Transgenerational Effects Following Exposure to Ionising Radiation

A briefing note prepared by a subgroup of the Advisory Group on Ionising Radiation

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EXECUTIVE SUMMARY

In this briefing note we review health effects in offspring of human populations exposed as a result of radiotherapy and some groups exposed to chemotherapy. We also assess risks in offspring of other radiation-exposed groups, in particular those of the Japanese atomic bomb survivors and occupationally and environmentally exposed groups. Experimental findings are also briefly surveyed.

Animal and cellular studies tend to suggest that the irradiation of males, at least at high doses (mostly 1 Gy and above), can lead to observable effects (including both genetic and epigenetic) in the somatic cells of their offspring over several generations that are not attributable to the inheritance of a simple mutation through the parental germ line. However, studies of disease in the offspring of irradiated humans have not identified any effects on health. The available evidence therefore suggests that human health has not been significantly affected by transgenerational effects of radiation. It is possible that transgenerational effects are restricted to relatively short times post-exposure and in humans conception at short times after exposure is likely to be rare. Further research that may help resolve the apparent discrepancies between cellular/animal studies and studies of human health are outlined.

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1 INTRODUCTION

Epidemiological studies to date have not provided clear evidence of heritable effects of radiation exposure in humans. Consequently, current genetic risk estimates for radiation are derived from measured germline mutation frequencies in male mice for a small number of marker genes, which have very low spontaneous and induced mutation frequencies (typically induced by radiation at about 1 in 100,000 per Gy of X-rays) [1, 2].

When it was reported [3] that the raised incidence of leukaemia and non-Hodgkins lymphoma among children living in Seascale near Sellafield showed a statistically significant association with paternal employment at Sellafield and the recorded external radiation dose prior to conception it triggered an avalanche of work on the possible health consequences in the offspring of fathers who had been exposed to radiation (paternal preconceptional irradiation, PPI). A review of subsequent work led the Committee on Medical Aspects of Radiation in the Environment (COMARE) to conclude in its 7th Report [4] that there was no convincing evidence to suggest that ionising radiation alone at the doses to which male nuclear industry radiation workers have been exposed results in an increased incidence of childhood cancer, a conclusion reached also by others [5-8]. In its 8th report COMARE also found “little epidemiological evidence that pregnancy outcomes in general are related to parental exposure to radiation. If there is an association, it is most likely a link between paternal (not maternal) radiation exposure and incidence of stillbirths and neural tube defects (spina bifida and anencephaly)” [9].

A number of reports suggested elevation in mutation frequencies in offspring of radiation-exposed groups. In particular, analysis of a Belarussian population exposed as a result of the Chernobyl nuclear accident suggested excess minisatellite mutations [10, 11]. A similar magnitude of excess risk was suggested in a population exposed as a result of the Kazakhstan nuclear weapons tests [12]. While these minisatellite mutations may be considered to be simple inherited mutations, they occur at a frequency that is much higher than expected when compared to conventional mutation frequencies, so they are therefore considered to be untargeted in nature (see for example [13]). However, no excesses of mini- or microsatellite mutations were observed in offspring of the Japanese atomic bomb survivors [14-17], nor in various other exposed groups [18-21]. Even when these excess mutation frequencies occur, it is not clear what relevance they may have for chronic disease, as discussed previously [13].

Set against that, a large number of animal studies suggested effects detectable in first-generation (F_1) offspring when moderate or high radiation doses to the father are employed. In particular, a number of studies of expanded simple tandem repeat (ESTR) mutations in F_1 offspring of irradiated male mice suggested excess frequencies that appeared to increase with increasing dose over the range of a few Gy, in partial contrast to some human studies with less apparent dose dependence, albeit from rather sparse data [13]. Very recent studies of male mice have presented further evidence that transgenerational effects may result from ionising radiation exposure [22, 23] as well as from commonly used chemotherapeutic agents [24]. Should such effects translate to

humans they would be of potential concern to men who have undergone radiotherapy and wish to have children. The Advisory Group on Ionising Radiation was asked to keep a watching brief on this area for the Health Protection Agency (HPA). In this briefing note for the HPA (which is now subsumed within Public Health England) we have therefore reviewed a number of these studies that seem to be particularly relevant and also summarised the available epidemiological evidence relating to health detriment in the offspring of people exposed to radiation. For the experimental animal studies we have focussed on those (i) in which fathers were exposed, (ii) where the estimated average testicular dose of low LET radiation was of the order of 0.1 Gy or above, (iii) and where the radiation was given as one or more acute exposures.

In contrast to the rare stable mutations, the animal studies that we review here imply that, in addition, preconceptional radiation may be capable of inducing subtle effects in the germline at very much higher frequencies and which can result in genomic instability in the first-generation (F_1) offspring and in subsequent generations. Such high frequency events are unlikely to be attributable to conventional targeted mutation events. We are aware that in the literature the term 'transgenerational' has been used in several differing senses. In this paper we consider that transgenerational effects elicited following irradiation of an F_0 male are those which **arise** in his descendents that are not: (i) attributable to inheritance of a conventional DNA mutation; or (ii) mutations arising in the next generation attributable to the transmission of damaged DNA through the sperm.. If they **arise** in the germ cells of the F_1 offspring they will be **expressed** as phenotypic effects in the F_2 and subsequent generations; alternatively they may be **detected** by direct examination of the germ cells in the F_1, e.g., by polymerase-chain reaction (PCR). If they **arise** in the somatic cells of the F_1 (or subsequent generation) offspring they may be **expressed** and **detected** in the same generation.

In theory, changes arising in the germ cells of the irradiated F_0 males may be expressed in any subsequent generation and are not, strictly speaking, transgenerational but heritable germ line mutations. Not all of the studies considered below were able to distinguish heritable mutation from transgenerational genomic instability. In particular, the studies reviewed here did not all assay effects in generations other than the first; indeed, there were only a few studies in which this was the case [25-31]. Other studies that provide evidence of transgenerational effects include those that show new mutations in the germ cells of the F_1 [32-36] or non-clonal mutations in somatic cells of the F_1 [32-35]. Certain endpoints that we considered, in particular foetal death, are not necessarily transgenerational. However, it is certainly possible that they are one expression of transgenerational effects, in particular transgenerational instability, and are therefore of relevance to the review. In Section 2 we review these indicative animal studies and any directly corresponding human counterparts; then in Section 3 we consider the epidemiological evidence for overt health effects that might result from such mechanisms in humans. In Sections 4 and 5 we summarise the experimental and epidemiological findings, and conclude with some recommendations for further research.

2 EVIDENCE INDICATIVE OF TRANSGENERATIONAL EFFECTS IN EXPERIMENTAL ANIMAL STUDIES AND HUMAN POPULATIONS

In this section we bring together work involving both laboratory animals and humans that examines evidence for effects in the progeny of irradiated fathers. These cellular effects are not health detriments in themselves but might indicate a potential for health effects in humans. Evidence relating to actual health effects in humans is summarised in Section 3.

2.1 Genetic Effects

2.1.1 Chromosome damage

In the F_1 and F_2 offspring of male Wistar rats given 3 Gy irradiation 25 days before mating (which the authors describe as the post-meiotic spermatid stage) with unirradiated females, chromosome aberrations were observed in hepatocytes stimulated to divide by partial hepatectomy; no aberrations were seen in the offspring of unirradiated fathers [29]. This study provides some evidence for transgenerational genome destabilisation although the results presented lack clarity and detail; furthermore, this remains an isolated report that requires confirmation by independent replication.

In BALB/c and CBA/Ca male mice given 1 Gy and 2 Gy X-rays, respectively, and mated 6 weeks after irradiation (and therefore at spermatogonial stage [37]) to unirradiated females of the same strain, there was an abnormally high level of DNA strand breakage in freshly taken bone marrow cells of the first-generation (F_1) male offspring, assayed using the alkaline Comet assay [38]. A highly significant two-fold increase in the number of γ-H2AX foci (which detect DNA double strand breaks) was also found in frozen spleen samples from first generation offspring of both strains. However, there was no significant difference between offspring of either strain of irradiated and control animals in levels of oxidised DNA bases, detected using the formamidopyrimidine glycosylase (FPG) Comet assay [38].

Tawn *et al.* [39] reported on the cytogenetic analysis of 43 offspring of parents identified from the Danish Cancer Registry who had survived radiotherapy for childhood cancer. The parents' gonadal doses varied widely, with paternal doses in the range 0.01-1.20 Gy and maternal doses in the range <0.01-9.20 Gy. Tawn *et al.* [39] found a lower frequency of chromatid aberrations or chromosomal gaps in the offspring of survivors with parental gonadal dose >0.05 Gy (mean 0.78 Gy) compared with offspring of survivors with lower gonadal dose (mean 0.02 Gy) (Table 1). However, results were not reported separately for exposed males; there being only 12 of these, there may have been limited power to detect adverse paternal-exposure effects.

Awa *et al.* [40] reported an extensive series of analyses of chromosome aberrations in the first generation (F_1) offspring of the Japanese atomic bomb survivors. There was no elevation in the number of children of exposed parents carrying chromosome

aberrations. The frequency of both sex chromosome aneuploidy and structural rearrangements was higher among the offspring of the control parents than among offspring of the exposed. However, no doses are reported for this study. As they screened only 10 cells from each offspring they would only have been able to detect aberrations present or arising in the germ cells of the parent and would be unlikely to have detected any arising as a result of genomic instability during development of the offspring, unless the effect was substantial.

Although not presented in Table 3 (because quantitative data were not given), 79 children born to Chernobyl clean-up workers (average exposure 231 mSv) were reported to have peripheral blood lymphocytes (PBLs) with an elevated frequency of chromosome aberrations when compared with children from unirradiated parents [41]. However, experimental details were scanty and there was lack of clarity concerning the composition of the controls used in the various parts of this study. A subsequently published study from the same group with 39 children from 31 families in which the father was a clean-up worker also found higher frequencies of aberrant cells in the offspring compared with a control group [33]. The time between exposure and conception varied from 1 month to 18 years but there was no apparent time dependence of aberrant cell frequency. Inappropriate statistical methods appear to have been used and, as with the previous study, there was a lack of clarity about the composition of the control group.

In summary:

- Evidence for an increase in DNA strand breakage in first-generation (F_1) offspring has been reported in two strains of mice exposed to high doses of X rays. Given that mating occurred six weeks after exposure very little if any DNA damage would have been present at the time of mating; the strand breaks observed in the F_1 may therefore be regarded as reflecting genomic instability.

- In rats chromosome aberrations were seen when hepatocytes of F_1 and F_2 offspring of fathers exposed to high doses of gamma rays were stimulated to divide. In contrast to the mice studies reported above, the relevant germ cells were irradiated at the post-meiotic spermatid stage. However, this is an isolated report, and requires independent verification.

- The effects observed in rats and mice are suggestive of genome destabilisation in the offspring of males exposed to high radiation doses; in rats the effect in the F_2, if confirmed, would appear to be transgenerational.

- Human studies, which involved average radiation doses lower than those given to animals, are inconclusive.

2.1.2 ESTR mutations in mice

A variety of studies have investigated frequencies of molecular changes in tandemly repeated DNA loci in mice and humans as potential indicators of mutational risk to the germline after radiation exposure (reviewed by Bouffler *et al.* [13]). In mice, unstable expanded simple tandem repeat (ESTR) loci show a very high spontaneous mutation rate in both germline and somatic cells, probably due to a mechanism of replication

slippage similar to that of microsatellite instability. Indeed, ESTRs are structurally similar to microsatellites, simply being longer (see for example [13]). Expansions of specific microsatellite sequences are known to be associated with several human genetic disorders [see, for example, the reviews of Mirkin [42] and Batra,*et al.* [43]] although no specific roles for ESTR expansions in disease are known. GC-rich minisatellites, in humans, consist of larger (9-60 bp) units that show considerable variation along the array. High-frequency mutation at minisatellite loci is almost completely confined to the germline and may be attributable to complex gene-conversion events. For both mouse ESTR and human minisatellites, mutation rates have to date been evaluated using either pedigree analysis (comparing tissue DNA from the first generation (F_1) offspring with DNA from the (F_0) parents) or, more recently, by single-molecule PCR (SM-PCR) approaches in sperm or somatic cells. The mutational behaviour of minisatellite loci differs dramatically from that of ESTRs and microsatellites, so the use of mouse ESTR loci as models for human minisatellite instability should be treated with caution [13].

As reviewed by Bouffler *et al.* [13], extensive pedigree-analysis studies in mice by several research groups (notably those of Dubrova (e.g., [44]), Niwa (e.g., [45]) and Boreham (e.g., [46])) have shown high frequencies of excess ESTR mutations in first generation (F_1) offspring (i.e., mutations induced in the F_0 germline) of male mice exposed to chemical mutagens [47] and ionizing radiations, including dose-dependent increases and strong dependence on the stage of spermatogenesis at the time of irradiation (although the latter dependence was not entirely consistent between studies). From the very high frequencies of induction it was concluded that mutations arise from some form of radiation-induced instability leading to non-targeted events in the irradiated germline. More recent studies, entirely from the Dubrova group, using pedigree analysis and/or SM-PCR analysis of the F_0 sperm, have confirmed and extended these results to a variety of additional biological and exposure conditions (including ENU exposure [48], *in utero* irradiation [49],, chemotherapeutic drug exposures [24, 50] and lower- dose and chronic irradiation [23]), and to ESTR mutations in somatic cells of the irradiated (F_0) mice [49]. Results from all of the above showed high-frequency induction of ESTR mutations in the directly-irradiated germline or somatic tissues. As explained in the Introduction, in this paper these F_0 germline mutations *per se* are considered to be not, strictly speaking, transgenerational effects, but rather heritable germ line mutations. Some of these studies, however, also included direct evidence of transgenerational effects, as discussed below.

Similar pedigree analysis methods have been applied to score *de novo* ESTR mutations that arise in the germline of the unirradiated first-generation (F_1) offspring of irradiated male mice and additionally in the germline of the next unirradiated F_2 generation. Early results for these unambiguously transgenerational effects were summarized by Bouffler *et al.* [13]. Most notably, following the pilot study of Dubrova *et al.* [51] with 0.5 Gy fission neutron irradiation of CBA/H mice, mated 10 weeks later with unirradiated females, Barber *et al.* [27] found that, in all of the three mouse strains tested (CBA/H, C57BL/6, BALB/c), there were approximately 2-3-fold increases in germline mutation rates in the unirradiated first (F_1) and second (F_2) generation offspring, of comparable magnitude to the raised germline mutation rates in the irradiated (F_0) fathers after pre-meiotic exposures to 2 Gy of X-rays or 0.4 Gy of fission

neutrons. In accordance with the methods of pedigree analysis, each of these increased germline mutation rates was detected as altered ESTR bands in the tissue of offspring compared to tissue from its parents. Additionally, the F_1 and F_2 germline mutation rates (as detected by raised mutation frequencies in the F_2 and F_3 offspring, respectively) were raised even after post-meiotic irradiation of male mice, under which conditions rates were not raised in the F_0 germline. The increased germline mutation rates were transmitted in the F_1 and F_2 generations equally through male and female lineages. The SM-PCR method was applied by Barber *et al.* [38] to confirm these results by direct detection of mutations in individual sperm of the first-generation (F_1) offspring after pre-meiotic irradiation (with 1 or 2 Gy of X-rays) of male F_0 mice of two strains (BALB/c, CBA/Ca, respectively) mated 6 weeks after irradiation and to detect similarly raised somatic-cell mutation frequencies in the F_1 bone marrow and spleen. [In this study increased mutation frequencies were reported also for the *hprt* protein-coding gene (see below) and for endogenous levels of single- and double-strand breaks in DNA (see above).]

Hatch *et al.* [52] confirmed that, even after post-meiotic irradiation of the F_0 germline, there were about 2-fold increases in ESTR mutation frequencies in the sperm and marrow of the unirradiated first generation (F_1) offspring mice. Results consistent with the above were obtained also in further X-ray investigations by Barber *et al.* [49] and Abouzeid Ali *et al.* [22], as well as generally similar results being obtained for other DNA-damaging agents (namely, ENU [48] and three chemotherapeutic drugs [24]). Following 1 Gy of X-rays *in utero* at 12 days of gestation and mating at 8 weeks of age, raised ESTR mutation frequencies were observed in the germline and somatic cells of the first-generation (F_1) offspring after paternal (F_0) *in utero* irradiation, but not after maternal *in utero* irradiation [49].

Mughal *et al.* [23] investigated the effects of lower doses of X-rays (acute) and of chronic low dose-rate X-ray exposure. Male BALB/c mice were exposed at the pre-meiotic stage acutely to 0.1, 0.25, 0.5 and 1 Gy of X-rays, or to 1 Gy delivered at low dose rate over two weeks. The SM-PCR method was used to measure ESTR mutation frequencies in sperm and brain of the irradiated (F_0) males and their first generation (F_1) offspring. Results for the F_0 males showed an approximately linear increase in mutation frequencies, with a doubling dose of about 0.6 Gy; there was a significant ~2-3-fold reduction in effect when the dose was delivered chronically. In the first-generation (F_1) offspring mutation frequencies were raised (by 2-2.5-fold relative to controls) only after 0.5 and 1 Gy acute irradiation of the fathers, but not at all after 0.1 or 0.25 Gy acute or 1 Gy chronic. Hence, the authors suggested that there was a threshold dose required to trigger the transgenerational instability and that the threshold is greater for chronic irradiation.

In summary:

- On the basis of two studies, radiation-induced ESTR instability following acute high dose irradiation of male mice is manifested in diploid cells after fertilisation and can be transmitted through both male and female germ lines for several generations. It would appear to be genuinely transgenerational and untargeted.

- These instabilities are manifest also in somatic cells (e.g., brain, bone marrow, spleen).
- One study has suggested the existence of a dose threshold for induction of ESTR mutations in F_1 offspring of irradiated male mice, but further work is needed to test the generality of this finding.
- There is some evidence that the transgenerational effects of maternal high dose acute irradiation are likely to be minimal.

2.1.3 Germline DNA changes in humans

The analysis of sperm DNA from three seminoma patients before and after radiotherapy failed to detect any increases in the frequency of minisatellite mutations. These men were exposed to 15 fractions of acute X-rays with a total testicular dose between 0.4 and 0.8 Gy [34].

Weinberg *et al.* [32] presented evidence of a large increase in new DNA mutations in the offspring of Chernobyl liquidators, suggestive of a genome destabilization effect. They used random-sequence PCR primers to detect putative mutations, a technique that has been questioned as being unvalidated [35] so it is unclear how robust this result is. To the best of our knowledge there have been no follow-up studies. It is not clear whether these DNA changes reflected artefacts in use of PCR, rather than real underlying changes in DNA sequence.

This contrasts with the study of Asakawa *et al.* [36] who assessed mutations in first-generation (F_1) offspring of 50 Japanese atomic bomb survivor families (66 children) with high mean gonadal dose (mean 1.7 Sv), and offspring of 50 control families (62 children); the distribution of maternal and paternal gonadal exposure is not specified. Asakawa *et al.* used a Restriction Landmark Genome Scanning (RLGS) assay to detect mutations, using a *Not*I marker enzyme. A single mutation was detected in the controls and none in the exposed group [36]. Given the low mutation frequency for this particular marker, this study has very little power to detect elevated risk. In the same paper they showed that RLGS mutations expressed in the F_1 offspring of irradiated mice also occur at a very low level, lower even than the rate estimated using the classic 7-locus specific locus assay [53].

In summary:

- From the limited amount of information available there is as yet no robust evidence from DNA studies for any induction of mutations (whether targeted or untargeted) in the germ cells of offspring of irradiated humans.

2.1.4 Somatic gene mutations in the offspring of exposed parents

Previous work had indicated that the offspring of irradiated male mice showed an increase in reversion of the unstable pink-eyed dilution p^{un} locus (coding for black coat colour spots and black eye spots) and that this occurred with both paternally-derived and maternally-derived alleles [54]. In this study male C57BL/6J p^{un}/p^{un} and C3H/HeJ p^{J}/p^{J} mice were irradiated with 1 – 6 Gy and directly mated with unirradiated females (so

that irradiation was at spermatozoal stage), also mated 15 weeks later (so that irradiation was at spermatogonial stage [37]). Significant effects were only seen in relation to spermatozoal irradiation. Similar results have been obtained with the Medaka fish [28, 55], in which male fish were irradiated with 0.64 – 9.5 Gy and mated 1-6 days later (corresponding to irradiation of sperm and late spermatids) or 1-3 months later (corresponding to irradiation, in part, of spermatogonia [37]). Some of the experiments also involved concomitant administration of ENU [55]. Mutations in the *wl* (*white leucophores*) locus (a pigmentation locus) were assayed. A significant increase in mutants was only seen after spermatid irradiation, and effects were also seen in the F_1 generation, but not the F_2 [28]. Following spermatozoal irradiation, the F_1 animals in all these studies would have inherited sperm DNA that had substantial DNA damage; it is likely that the somatic mosaicism in these three studies resulted from fixation of mutation in the first few embryonic divisions, so that these are not likely to be transgenerational (i.e., reflecting genome destabilisation) *sensu strictu*.

Barber *et al.* [38] measured the frequency of *hprt* (thioguanine resistant) mutants in splenocytes from first generation offspring from irradiated male mice mated six weeks after exposure. Unlike the p^{un} locus, the *hprt* gene has normal stability with a low mutation rate in both somatic and germline cells. BALB/c and CBA/Ca mice were given 1 Gy and 2 Gy of X-rays, respectively. A highly significant 3.3-fold and 3.7-fold increase in the frequency of *hprt* mutants was found in these strains, respectively. The increase was observed in all offspring of irradiated males. The authors pointed out that the X-linked *hprt* locus in first generation offspring was inherited from the unexposed mothers, which implies a genome-wide destabilisation after fertilisation. The increase reported therefore should reflect untargeted mutations arising from genome instability.

The *hprt* assay is readily applicable to human peripheral blood lymphocytes but we have been unable to find any study in which the frequency of thioguanine-resistant mutants has been examined in the lymphocytes of the offspring of irradiated fathers.

In summary:

- Mouse studies indicated a raised frequency of untargeted gene mutations in somatic cells of offspring of males given 1 or 2 Gy X-rays; there appear to be no comparable human data.

2.1.5 Minisatellite mutations in humans

When this field was reviewed under the aegis of COMARE [13] there were a number of studies on human populations, most of which had uncertainties concerning dosimetry and/or possible confounding factors.

Studies with the children of A-bomb survivors [14-17, 36] were uniformly negative. In the most recently reported analysis, data on a total of 40 minisatellite loci failed to show any sign of an increase in minisatellite mutation rate in the offspring of irradiated parents [16].

The children of cancer survivors who received radiotherapy constitute another population which has recently received attention [56]. DNA samples from 100 families, where one parent was a cancer survivor, were analysed for mutations at 8 hypervariable

minisatellite loci by Southern hybridisation. Gonadal doses in this study were substantial, with mean paternal testicular doses of 1.23 Gy and mean maternal ovarian doses of 0.58 Gy; some cancer survivors had also received chemotherapy. No significant difference was found between the mutation frequency of 5.6% in exposed fathers and that of 5.8% in unexposed fathers. The mutation rate of 1.6% in exposed mothers was not significantly different from 2.1% in unexposed mothers. Subgrouping the exposed fathers into dose groups of <0.10 Gy, 0.10-0.99 Gy, 1.00-1.99 Gy, ≥ 2.00 Gy revealed no significant differences in mutation frequencies compared with unexposed fathers. There were no differences in mutation frequencies associated with treatment with chemotherapeutic agents.

The families of Chernobyl clean-up workers have been subject to four large studies of minisatellite or microsatellite mutation rates all of which failed to observe any increase associated with paternal exposure [18-21]. In particular, the Ukraine liquidator study of Livshits *et al.* [21] considered the offspring from fathers during or up to two months after working as cleanup workers (subgroup 1: 88 children) or at least 4 months after (subgroup 2: 95 children), with paternal doses ranging from 0.028 to 1.2 Sv, as well as a control (unexposed) group from southern Ukraine, again split before (43 children) and after (120 children) the accident. There were no elevated minisatellite mutation frequencies overall (exposed *vs* unexposed) (p>0.1), or differences between the two exposed subgroups (p>0.2) [21] (Table 3). The Estonian liquidator study of Kiuru *et al.* [18] considered liquidators with children born either side of the fathers having worked at the Chernobyl accident site (between 1986-1991), and studied 148 pre-Chernobyl and 198 post-Chernobyl children (the latter children had to be born within 33 months of the father having worked at the Chernobyl site). Paternal doses ranged from 0.043 to 0.300 Sv. There were no significant findings overall, although there was a borderline significant increased odds ratio, 3.00 (95% CI 0.97, 9.30) in the highest paternal dose group (0.2-0.3 Sv) [18] (Table 3). The Ukraine study of Slebos *et al.* [19] analysed mini- and microsatellites in families with children conceived before and after the fathers' work at the Chernobyl accident site between 1986 and 1990, analysing a total of 80 families for these endpoints. There were no significant differences between the groups, whether for mini- or microsatellite markers (p>0.1). The median dose was 0.15 Sv, although detailed dose information, or on the timing of exposure in relation to conception, was not given. The Belarus study of Furitsu *et al.* [20] examined offspring of 64 liquidator families (in two cases both father and mother were liquidators, in another only the mother), working at the Chernobyl site in 1986 or 1987, The children were conceived before and after their parent's work as liquidators, and children of 66 unexposed control families were also studied. There was no information on parental exposure history and little on timing of conception in relation to exposure; most children of liquidators were born in 1987, although one was born in 1983, and six others in 1988-1992. Children of the controls were born in the period 1985-1989. There were no significant variations in microsatellite frequencies between exposed and control groups (p>0.3) (Table 3) [20].

There is, however, evidence of an increase in minisatellite mutations in offspring of various groups exposed as a result of environmental contamination. Dubrova *et al.* reported excess minisatellite mutations in a Belarussian population exposed as a result of the Chernobyl nuclear accident, receiving doses of about 28 mSv [10, 11]; however, interpretation of this study was substantially limited by its use of a possibly non-

comparable control group from the UK. A similar magnitude of excess risk (about twofold, confined to paternal exposures) was suggested in a subsequent study, with more appropriate controls, in a similar Chernobyl-exposed cohort in Ukraine [57] exposed at similar levels (<50 mSv) [57]. Similar magnitudes of risk were observed also in a population exposed as a result of the Kazakhstan nuclear weapons tests [12], exposed to doses in excess of 1 Sv (although there was no elevation in the later part (1961onwards) of the F_2 generation), and in a Techa-river exposed group [58], with paternal and maternal exposures of 0.102 and 0.086 Sv respectively. It is notable that one of the A-bomb studies [15] used the same probes as used by Dubrova *et al.* The mean paternal gonadal doses in the exposed group of 30 fathers was 1.34 Gy, and among the 32 exposed mothers it was 1.6 Gy [15], and therefore very much higher than the mean gonadal doses in the studies of Dubrova *et al.* [10-12, 57, 58]. In these positive studies of Dubrova *et al.*, as also in the negative Chernobyl liquidator studies [18-21], but in contrast to the atomic bomb survivor and radiotherapy studies mentioned above, exposures were protracted and included significant components from internal radionuclides [59, 60]. Among the problems in dealing with these data are the difficulty of allowing for confounding factors such as chemical pollution, the fact that both parents were exposed and the absence of robust individual radiation dosimetry.

On the basis of work with mice, Mughal *et al.* [23] have suggested that there may be a threshold for the induction of ESTR mutations following paternal irradiation, and that this could explain the failure of some human studies to show induction of minisatellite mutations. However, their explanation rests on the assumptions that: (a) there was direct correspondence between the mutational behavior of mouse ESTR and human minisatellites (a questionable assumption – see Bouffler *et al.* [13]); and (b) that in radiotherapy a single exposure rarely lead to a testicular dose exceeding 0.1 Gy; and (c) that total fractionated treatments were generally around 1 Gy. While this might be true for current practice, it does not appear to correspond with the data in the available epidemiology studies where past practice exceeded these doses.

In summary:

- Although there are unresolved questions, there is a weight of evidence that acute high dose paternal exposures have not led to detectable increase of minisatellite mutations in the offspring of humans.
- It is not possible to make such a conclusion with regard to protracted internal exposures or mixed internal/external exposures where confounding factors, lack of robust individual radiation dosimetry and other problems hinder analysis.

2.2 Induced radiosensitivity

An increased sensitivity to the induction by radiation of chromosome aberrations in liver cells of the first-generation (F_1) offspring of irradiated male rats was reported by Vorobtsova [61]. This was also found in bone marrow cells and foetal fibroblasts [62]; a similar increase in sensitivity to cyclophosphamide was also reported. In contrast, Slovinska *et al.* [29] did not observe any increase in the radiosensitivity of cytogenetic damage in the first (F_1) and second (F_2) generation offspring of irradiated rats using

doses of 3 Gy. In these experiments mating occurred 25 days after irradiation so that the transmitted germ cells would have been at the post-meiotic spermatid stage [37]. In the experiments of Vorobtsova F_0 male rats were mated with two females over a period of 3-4 days after irradiation with 4.5 Gy, so the transmitted germ cells would have been exposed as spermatozoa [37]. The dose (4.5 Gy) resulted in around 30% dominant lethals. The difference between the results of Vorobtsova and Slovinska *et al.* may perhaps be attributed to an ability of spermatids to repair damage that is refractory to repair in spermatozoa.

Interpretation of results of studies with the offspring of cancer patients who have survived and reproduced following radiotherapy is complicated by the evidence that chromosomal radiosensitivity is associated with cancer-proneness [63, 64]. Any increase in chromosomal sensitivity in the offspring of patients who had radiotherapy before conception of their children could thus be attributed either to the effect of the radiotherapy or to inherent radiosensitivity associated with cancer-proneness (inherent here taken to embrace genetic and/or environment and lifestyle factors). Chromosomal radiosensitivity has been reported in cancer patients in studies undertaken before treatment as well as studies undertaken some time after treatment, thus disputing suggestions that it is treatment induced [65]. Moreover specificity of G_2 chromosomal radiosensitivity to inherited predisposition to cancer is suggested by the finding of a lack of enhanced radiosensitivity for two cancer sites, lung and cervix, primarily associated with environmental and lifestyle factors rather than heritable factors [66].

These possibilities need to be considered in the interpretation of results reported by Vorobtsova *et al.* [67] for PBLs cultivated from children born after radiotherapy and chemotherapy was given to their parents. With doses of 0.25-1.5 Gy gamma radiation to the lymphocytes there was an increased number of chromosome aberrations in metaphases [67]. However, for only two of the 14 children was the irradiated parent the father. Moreover, there are problems of clarity in both experimental procedure and analysis.

These considerations are also relevant to the study of Curwen *et al.* [68], that examined G_2 chromosomal radiosensitivity in a group of 23 Danish survivors of childhood and adolescent cancer, a control group comprising their partners and a group of 38 of their offspring (Table 1). This study suggested a substantial (and statistically significant) heritable component to G_2 radiosensitivity, with Mendelian autosomal dominant transmission [68]. There was statistically significant elevated radiosensitivity of survivors with respect to cutoffs defined by an external control group (drawn from the investigators' laboratory, although this control group was not assessed completely concurrently), but there was none with respect to cutoffs defined by the control group of partners of the cancer survivors (Table 1). However, it is difficult to draw conclusions from this study because of differences between the two control groups and the small numbers. A follow-up study by Curwen *et al.* [69] in the same group also failed to demonstrate differences in radiosensitivity with respect to cutoffs defined by a partner control group (Table 1). There was no external control group in this follow-up study. Curwen *et al.* [69] used the 90% percentile of the patients' partners as the cut-off point. Although the proportion of individuals displaying enhanced radiosensitivity was twice as high in both the cancer survivor and offspring groups than the partner controls, neither

reached statistical significance, consistent with the previous study. If there is an increase in chromosomal radiosensitivity in offspring of cancer patients of the sort weakly suggested by the work of Curwen *et al* [68] this is most likely to be the result of inheritance of the radiosensitivity trait seen in their parents.

In principle the Chernobyl clean-up workers constitute a cohort that was not (unlike the radiotherapy cohorts) selected for cancer-proneness and their offspring would not be expected to have inherited radiosensitivity from their parents. Aghajanyan and Suskov [41] report that the PBLs of children of Chernobyl-liquidator fathers were more sensitive than those from the children of unexposed fathers to the induction of chromosome aberrations after administration of 0.2 and 0.3 Gy *ex vivo*. However, experimental details were scanty and this group also included children whose parents have been exposed as a result of living in contaminated areas which makes interpretation of this study problematic. Little reliance can be placed on this study.

In summary:

- There is no convincing evidence from human studies that exposure of men to radiation at doses of the order of a few hundred mGy causes increased sensitivity to radiation in their offspring. To detect modest increases in radiosensitivity, especially in view of the inherited predisposition among cancer patients, much larger studies would be required.
- One study in which increased radiosensitivity was reported in the offspring of irradiated male rats employed much higher (around 10x) paternal doses. Whereas in the human studies the germ cell stages irradiated would have been spermatogonia or stem cells, in the rat study they would have been exposed as spermatozoa and could have contained unrepaired DNA damage at the time of conception.

2.3 Cell proliferation defects

It has been recognised for many decades that microorganisms and cultured cells that survive radiation doses above, say, 0.5 Gy frequently show a decreased rate of proliferation. This may also occur in the whole animal. Preimplantation aggregation chimeras have been exploited in a series of studies in which the relative proliferative ability of embryonic cells with a radiation history was compared with cells with no such history. To test paternal germ cell irradiation for transmission of embryonic effects male mice were irradiated and bred once weekly for 9 weeks postirradiation to evaluate the response of progressively earlier stages of spermatogenesis from mature sperm (week 1 postirradiation) to spermatogonial stem cells (weeks 8,9 postirradiation). In the first study [70] there was a proliferation deficit with cells with a paternal radiation history (0.05 Gy, 0.17 Gy, or 1.73 Gy) that peaked at week 7. In a subsequent more detailed study [71] male mice were briefly irradiated with ^{137}Cs gamma rays at nominal absorbed doses of 0.0, 0.0015, 0.005, 0.010, or 0.05 Gy and then mated for the next 8 weeks to untreated females. Significant decreases in proliferation ratios were observed at postirradiation weeks 4, 6, and 7 for the 0.01-Gy dose group and at weeks 5-6 for the 0.05-Gy dose group.

Subsequent work showed that the deficit in competitive cell proliferation persisted without degradation in the second (F_2) generation of embryos when F_0 males received 1.0 Gy gamma radiation 6 and 7 weeks prior to conception of the first-generation (F_1) males [30]. The proliferative deficit observed when type beta spermatogonia were irradiated appears to be found in male germ cells as well as embryonic cells as evidenced by germline drift experiments to the second (F_2) generation [26].

Experiments aimed at elucidating the mechanism of the proliferative deficit in mice have indicated that the effect was blocked by an inhibitor of gap junction intercellular communication [72], and was associated with alterations in gene expression that persisted into the third (F_3) generation offspring [31]. Other experiments have detected changes in comet assays on third-generation (F_3) sperm descended from F_0 irradiated type beta spermatogonia, changes which were increased by ATM heterozygosity [25].

Slovinska *et al.* [29] observed cell proliferation in rats that had undergone partial hepatectomy. Hepatocytes in the descendents of male Wistar rats that had received 3 Gy 25 days before mating (i.e., at post-meiotic spermatid stage [37]) showed a reduced proliferating activity compared to unirradiated rats and this was associated with a higher frequency of chromosomal aberrations and a higher proportion of cells with apoptotic DNA fragments. Similar, though less pronounced, changes occurred in regenerating rat liver cells in the first (F_1) and second (F_2) generation offspring of irradiated male rats.

In summary:

- A deficit in cellular proliferative activity was found in the offspring of irradiated male mice persisting for several generations; limited work with rats was consistent with this. The effect may be regarded as transgenerational and likely to involve persisting gene expression (epigenetic) changes.

2.4 Transgenerational induction of cancer

The work of Nomura [73] reported a substantial increase in the incidence of tumours in the first-generation (F_1) offspring of three strains of mice exposed to X-rays. However, subsequent work following Nomura's protocols failed to confirm the result in two internationally-available strains of mice (BALB/cJ and C3H/HeH) and showed a temporal (possibly seasonal) variation in tumour incidence in mouse colonies [74, 75], which offered a possible explanation for the reported differences. The failure to use contemporary controls is a potential problem in animal work, particularly as such detail was rarely documented. There do not appear to have been more recent reports showing increased tumour incidence in first-generation (F_1) offspring of irradiated mice but there are several papers reporting increased tumour incidence or altered tumour type when such offspring were exposed to other carcinogens [76-79], although not all such results have been positive [75]. It has also been reported that exposure of male mice to chemical carcinogens can increase cancer incidence in their progeny [80, 81].

In summary:

- If sensitivity of the F_1 generation to carcinogen exposure is real, it is presumably due to epigenetic alteration. It does not, however, seem to be a general phenomenon following paternal irradiation.

2.5 Congenital malformations

Extensive studies by Nomura assayed dominant lethal mutations and congenital malformations amongst the progeny of male or female mice of several different strains exposed to radiation or chemical mutagens [73, 76, 82-84]. The evidence of increased incidence of congenital malformations in the first-generation (F_1) offspring of X-ray or chemically exposed male mice was novel and was subsequently verified with X-rays [85, 86] and with chemical mutagens [87-91]. It is not clear whether these effects are transgenerational.

In summary:

- Congenital malformations and dominant lethals can be induced by paternal and maternal irradiation (and chemical exposure), but it is not known whether these effects are transgenerational.

2.6 Possible mechanistic contributors to transgenerational effects

As noted in 2.1.1 above, some studies indicate that higher levels of DNA damage were observed in the somatic tissues of first-generation (F_1) offspring of irradiated male experimental animals [29, 38, 92]. The origin of such DNA damage is not clear but its presence could contribute to the chromosomal aberrations and mutations that have been observed.

In the cell proliferation studies (Section 2.3) altered expression of some proteins involved in the control of cell proliferation (e.g. PKC, MAPK, p53, p21) has been documented [31, 92, 93]. These protein expression studies suggest that there may be some alterations to gene expression in the offspring of irradiated parents. Persistent alteration of gene expression can be caused by so called ‘epigenetic effects’ which include methylation of cytosine residues in DNA, modification of histones and regulation by micro-RNA expression. Barber *et al.* [49] have suggested that epigenetic phenomena may underlie the transgenerational ESTR mutation observed in mice. Radiation exposure has been shown to affect DNA methylation [94], histone modification [95] and micro-RNA expression [96, 97]. A potential role for epigenetics in transmissible instability has been identified by Filkowski *et al.* [98]. Exposure of male mice to 2.5 Gy X-rays 4 days before mating led to reduced methylation of certain repetitive DNA sequences in thymus tissue of offspring. The offspring of irradiated males had reduced levels of lymphoid specific helicase (LSH) in thymus tissue. These effects in the offspring were attributed to upregulation of micro-RNAs miR29 and miR296 in the irradiated father’s germ line leading to decreased expression of DNA

methyltransferase Dnmt3a, one of the enzymes that methylates cytosine residues. It should be noted that as the assays were all on the whole testis is it unclear whether it was actually germ cells in which the miRNAs were disregulated. Further work is required to establish the mechanisms that contribute to transgenerational effects, in particular the precise relation (whether causal or not) between the persistent elevation of DNA damage and the persistent epigenetic alteration of gene expression.

3 EPIDEMIOLOGICAL EVIDENCE FOR TRANSGENERATIONAL HEALTH EFFECTS

The data to be discussed are all presented in Tables 1-3. We discuss endpoints approximately in order of impact on health; they are also ordered in roughly this way in the Tables.

A general problem with many of the studies of offspring of cancer survivors that we consider in this Section is that radiation dosimetry was poor. In many cases information was only available on cancer survivors as a whole (without knowledge of exposure) [68, 99-101], so that one cannot infer much about the transgenerational effects of radiation. In some other studies the information on radiation exposure used in the analysis was limited, generally confined to employment of approximate parental gonadal dose groups [102, 103].

3.1 Cancer and other chronic diseases

As noted above, certain cancers are known to have a genetic component so that the offspring of cancer patients may also be cancer prone, something that would be a confounding factor in studies where cancer patients have had radiotherapy prior to fathering children. Nevertheless, Table 1 suggests that there are few indications of adverse effects, be they cancer or other chronic diseases, in offspring of most radio-therapeutically exposed populations.

In particular, there were no indications of excess cancers in offspring of the Danish Thorotrast patients [104], nor in groups of Finnish [100] and UK [99] pediatric cancer survivors (Table 1).

Table 2 suggests that there were few indications of adverse effects in offspring of Japanese atomic bomb survivors. Little [105] observed indications of excess risk for respiratory disease mortality in the first-generation (F_1) offspring cohort, but for no other endpoints (among 7 causes of death examined) were there any such indications of excess risk, and there were no indications of excess mortality or cancer incidence overall. Other analyses revealed few indications of excess risk for all malignant endpoints, whether for mortality or morbidity, and all other mortality endpoints [105-110] (Table 2). There were no indications of radiation-associated increases in adult-onset multifactorial disease [111, 112] (Table 2).

Table 3 suggests that there were few indications of adverse cancer effects in offspring of most occupationally and environmentally exposed populations. Perhaps the strongest evidence for adverse effects are to be found in the Nuclear Industry Family Study of Roman *et al.* [113]. However, this study is methodologically problematic, as it relied for ascertainment of cancer incidence on self-reports by the parents. There were no similar indications of excess risk in the study of offspring of a very similar population of UK nuclear workers by Draper *et al.* [8] that relied on register-based methods of case ascertainment and ascertainment of occupational exposure.

In summary:

- There is little evidence to suggest that the offspring of fathers or mothers who have been exposed to radiation are more prone to cancer or to any other chronic diseases.

3.2 Congenital malformations

There were no indications of excess congenital malformations in offspring of four groups of paediatric cancer survivors [99, 101, 103, 114, 115] (Table 1). In particular there was no suggestion of dose response for congenital abnormalities in a US group of childhood cancer survivors [115] (Table 1).

Table 2 suggests that there were no indications of adverse effects on congenital abnormalities in offspring of Japanese atomic bomb survivors [116]. It has recently become apparent that because of inadvertent omission of some data records, an updated analysis of this dataset [117] was flawed (RE Shore personal communication). There are no occupationally or environmentally radiation-exposed groups in which congenital abnormalities were assessed (Table 3).

In summary:

- Excess congenital malformations have not been detected in the offspring of irradiated fathers and mothers.

3.3 Stillbirth and preterm birth

Perhaps the strongest evidence for adverse effects in the cancer-treated group are to be found in relation to stillbirth and neonatal death in offspring of US childhood cancer survivors for maternal exposure before menarche [118], although there was no risk for maternal exposure after menarche. Signorello *et al.* [118] interpreted their finding as representing an effect of radiation on the uterus rather than on the ovaries (oocytes). Interestingly, there was also a raised risk of spontaneous abortion in offspring of a group of female Danish childhood cancer survivors [119]. However, there was only very limited radiation (or chemotherapeutic) dosimetry in the Danish group, based on assignment of survivors into four gonadal dose groups (low, low or medium, medium or high, high), so it is not clear whether this reflects radiation treatment or some concomitant (e.g, chemotherapeutic) exposure.

Table 2 suggests that there were no indications of an excess of stillbirths or neonatal deaths in offspring of Japanese atomic bomb survivors [117].

Parker *et al.* observed indications of excess stillbirth risk in offspring of Sellafield nuclear workers [120], but no similar excess is seen in the Japanese atomic bomb survivor first-generation (F_1) offspring cohort [121]. As discussed by Little [121], there are certain features of the analysis by Parker *et al.* [120] that may result in bias, in particular use of national data to estimate effects of paternal age in the Sellafield cohort. As also discussed by Little [121], certain of the models used by Parker *et al.* [120] do not employ adequately smooth likelihoods, so that asymptotic convergence (and hence accuracy of the stated confidence intervals) is not guaranteed. Abrahamson and Tawn [122] examined the incompatibility of the findings of Parker *et al.* [120] with those from other epidemiological and experimental investigations and suggested that the lack of consideration of a number of background factors affecting stillbirth rate, especially those relating to the mother, may have influenced the findings.

In summary:

- There is no reliable evidence for an increase in stillbirths among the offspring of irradiated fathers and mothers.

3.4 Sex ratio

There were no indications of effects of radiation exposure to either parent markedly affecting sex ratio in the offspring of a group of Danish paediatric cancer survivors [102], Japanese atomic bomb survivors [123], or UK radiation workers [124]. There are no occupationally or environmentally radiation-exposed groups in which the sex ratio was assessed (Table 3).

3.5 Birthweight

There were no indications of effects of radiation exposure to either parent markedly affecting birthweight in the offspring of a group of Danish paediatric cancer survivors [125]. There were also few indications of birthweight effects in offspring of women given diagnostic radiation exposures for scoliosis in comparison with a general population reference group, although there were indications of decreased birthweight when the lowest quartile of the scoliosis cohort was used as a reference group [126].

Analysis of birthweight in the offspring of the Japanese atomic bomb survivors in relation to parental gonadal dose has been subject to a limited chi-square heterogeneity analysis in relation to parental gonadal dose group, which suggested borderline significant evidence of heterogeneity ($\chi^2_{15} = 27.07$, $P[\chi^2_{15} \geq 27.07] = 0.03$) [127]. The authors indicated that “the statistical significance apparently results primarily from a higher frequency of immature infants (with birth weights of <2.5 kg) in the ≥1.00 Sv dose group than in the 0 Sv dose group” [127].

4 DISCUSSION

The cellular and animal studies raise a number of questions about their implications for human exposure that need to be addressed (particularly with respect to children born to men who have survived radiotherapy).

There is good evidence in mice and rats that paternal irradiation before conception resulted in a proliferative deficit observable in aggregation chimeras that persisted for several generations [26, 30, 70, 71]. The effect appeared to have an epigenetic basis and seemed to tail off (in mice) for matings around 8 weeks after exposure, so that the results would be unlikely to apply to men conceiving after radiotherapy where average times would be expected to be very much longer. Possible detriments attributable to proliferative deficit might include congenital malformations, spontaneous abortions, and stillbirths but the human epidemiology provides little support for such an effect. Perhaps the most sensitive marker might be birth weight, but although studies of this endpoint were generally negative [125, 126], there were borderline significant indications of an effect of birthweight in the Japanese atomic bomb survivors first-generation (F_1) offspring cohort [127]. It should be noted that the effects in rats were observed for matings within the first 7 weeks after irradiation and observations at such early times are largely absent from the epidemiological data. If, as postulated, the effects in rodents are mediated by epigenetic mechanisms, the persistence of such effects, should they occur in humans, is unknown.

Work with rats using high-dose preconceptional irradiation at the spermatozoa stage confers enhanced radiosensitivity to cells in the offspring [61, 62]. This was not observed when irradiation occurred at premeiotic spermatid stages [29] so one would not expect effects to occur in the offspring of radiotherapy patients. Nevertheless, some results with cells from such children have been interpreted as indicating enhanced chromosomal radiosensitivity [67]. Another, perhaps more likely interpretation would be that a significant proportion of cancer patients were chromosomally radiosensitive *per se* and that this property tends also to be manifest in their children. While it is not possible to give a definitive conclusion on this, it seems likely that, should there be any detriment, it would only occur if the children were themselves exposed to radiation or other genotoxic agents.

It should be noted that several studies of chemical exposures, in particular those of Skinner *et al.*, suggested the existence of transgenerational effects over at least three generations of offspring, for example after exposure to endocrine disruptors [128], fungicide [129] and dioxin [130].

Work with rats suggested that the offspring of irradiated males showed an enhanced level of spontaneous chromosomal damage [29], and DNA strand breakage (which would be expected to result in chromosome damage) has also been reported in offspring of irradiated male mice [38]. The work of Tawn *et al.* [39] with the children of cancer survivors did not indicate any increase in chromosome damage and was apparently at odds with the results of work with Chernobyl clean-up workers [41], although this latter study appears to be unsound.

The strongest evidence for induction of genetic damage in offspring comes from studies of ESTR mutations and somatic gene mutations in mice. The epidemiological picture is more mixed. Minisatellite mutations were not observed to be induced by external acute irradiation in the offspring of cancer survivors, of A-bomb survivors [14-17], or by the relatively protracted radiation exposure (from a mixture of internal and external source) received by Chernobyl clean-up workers [18-21]. The contrasting results from these minisatellite mutation studies of Chernobyl clean-up workers (liquidators) and those living in regions contaminated by the Chernobyl accident [10, 11, 57] are very hard to reconcile. Exposures would generally have been to similar contaminants with liquidators commonly receiving higher exposures over shorter periods; radiation dose protraction and reduction is most commonly associated with smaller effects.

Overall one might expect that if there were enhancement of radiation-induced genetic damage in the cells of children born to irradiated fathers, one area of potential concern would be malignant disease. However, most epidemiological studies have failed to find any elevated risk. If there were any increased risk of cancer it must be very small under the variety of situations studied.

The observed differences between the results of studies of animals and humans have yet to be resolved. One factor that may explain part of the discrepancy may be the substantial difference in time-scales. For example, in the experimental studies, rodent offspring were conceived within a few months of paternal irradiation whereas conception of human offspring following radiotherapy of the fathers was typically measured in years. The effects in rodents have been attributed to epigenetic changes and the prolonged post-irradiation period in humans before conception may allow such effects to be ameliorated. However, this does not explain why effects have been observed in certain environmentally exposed groups but not in other groups exposed in a similarly protracted fashion.

5 CONCLUSIONS

Animal and cellular studies tend to suggest that the irradiation of males, at least at high doses (1 Gy and above), can lead to observable effects (including both genetic and epigenetic) in the somatic cells of their offspring over several generations that are not attributable to the inheritance of a simple mutation through the parental germ line. However, studies of disease in the offspring of irradiated humans have not so far identified any effects on health, possibly in part a result of lack of statistical power. The available evidence therefore suggests that human health has not been significantly affected by transgenerational effects of radiation. As noted earlier any transgenerational effects may be restricted to relatively short times post-exposure and in humans conception at short times after exposure is likely to be rare.

The discrepancies between cellular/animal studies and studies of human health are striking and deserve clarification. Further research that may help resolve the apparent discrepancies between cellular/animal studies and studies of human health include:

(i) studies of *hprt* gene mutation frequencies in the lymphocytes of offspring of radiotherapy survivors – an association with paternal radiation exposure has been reported in mice but no human data exist;

(ii) more extensive searches for non-clonal chromosomal aberrations in lymphocytes of the offspring of Japanese atomic bomb survivors and other exposed groups where exposures are well documented – the available data are not able to rule out an association;

(iii) more extensive surveys of the occurrence of still birth, low birth weight and other untoward pregnancy outcomes in the offspring of irradiated fathers – the available data are scanty; and

(iv) further studies of minisatellite mutation in radiation-exposed human populations, particularly with internal and/or protracted exposures and where it is possible to minimise confounding factors.

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APPENDIX A TABLES

Table 1 Risks in offspring of diagnostically- and therapeutically-exposed patients. All reported risks use values from the original reports

Reference	Endpoint	Risk (and 95% CI)
Little *et al*. [104]	Leukaemia incidence in offspring of Danish Thorotrast-exposed patients	<-0.3 (<-0.3, 7.3)[a]
	Leukaemia and non-Hodgkin's lymphoma incidence in offspring of Danish Thorotrast-exposed patients	<-0.3 (<-0.3, 5.6)[a]
Madanat-Harjuoja *et al*. [100]	Risk of cancer in offspring of Finnish pediatric and early onset cancer survivors	1.67 (1.29, 2.12)[c]
	Risk of cancer in offspring of Finnish pediatric and early onset cancer survivors, excluding 54 hereditary cancer syndromes	1.03 (0.74, 1.40)[c]
Hawkins *et al*. [99]	Mortality from congenital abnormalities in offspring of UK children treated for leukaemia and non-Hodgkin's lymphoma	1.35 (0.03, 7.54)[b]
	Mortality from malignant neoplasms in offspring of UK children treated for leukaemia and non-Hodgkin's lymphoma	0.00 (NA, NA)[b]
	Mortality from all other causes (than congenital abnormalities and malignant neoplasms) in offspring of UK children treated for leukaemia and non-Hodgkin's lymphoma	2.30 (0.84, 5.02)[b]
	Mortality from all causes in offspring of UK children treated for leukaemia and non-Hodgkin's lymphoma	2.00 (0.80, 4.11)[b]
Winther *et al*. [103]	Congenital malformations in offspring of Danish childhood cancer survivors: hazard ratio (with respect to sibling offspring) of offspring of non-irradiated parent	1.0 (0.8, 1.4)
	Congenital malformations in offspring of Danish childhood cancer survivors: hazard ratio (with respect to sibling offspring) of offspring of irradiated parent	1.2 (0.9, 1.8)
	Congenital malformations in offspring of Danish childhood cancer survivors: hazard ratio (with respect to sibling offspring) of offspring of irradiated parent by gonadal dose group	Low[g]: 1.7 (1.1, 1.9) Low-medium[g]: 1.5 (0.7, 2.9) Medium-high[g]: 0.9 (0.3, 2.7) High[g]: 0.8 (0.3, 2.5)
Winther *et al*. [114]	Genetic disease (chromosomal abnormalities, congenital malformations, stillbirths, neonatal deaths) in offspring of Danish childhood cancer survivors	Unirradiated (referent): 1 Irradiated: 1.02 (0.59, 1.44)[d] No chemotherapy (referent): 1 Alkylating drug: 0.82 (0.53, 1.28)[d] No chemotherapy or radiotherapy (referent): 1 Alkylating drug: 0.75 (0.26, 2.13)[d]
	Genetic disease (chromosomal abnormalities, congenital malformations, stillbirths, neonatal deaths) in offspring of Danish childhood cancer survivors in relation to paternal testicular dose	0 Gy (referent) 1[d] 0-0.49 Gy 0.84 (0.48, 1.49)[d] ≥0.50 Gy 1.12 (0.44, 2.88)[d]
	Genetic disease (chromosomal abnormalities, congenital malformations, stillbirths, neonatal deaths) in offspring of Danish childhood cancer survivors in relation to maternal ovarian dose	0 Gy (referent) 1[d] 0-0.49 Gy 1.12 (0.52, 2.38)[d] ≥0.50 Gy 1.04 (0.17, 6.25)[d]
	Genetic disease (chromosomal abnormalities, congenital malformations, stillbirths, neonatal deaths) in offspring of Danish childhood cancer survivors in relation to maternal uterine dose	0 Gy (referent) 1[d] 0-0.49 Gy 1.34 (0.77, 2.32)[d] ≥0.50 Gy 2.30 (0.95, 5.56)[d]

Signorello *et al*. [115]	Congenital malformations in offspring of US childhood cancer survivors: relative risk of offspring of irradiated fathers (with respect to unirradiated survivors), testicular dose	Referent: 1 0.01-0.09 Gy 0.71 (0.31, 1.63)[i] 0.10-0.49 Gy 0.88 (0.33, 2.36)[i] ≥0.50 Gy 1.01 (0.36, 2.83)[i] p-value trend 0.90
	Congenital malformations in offspring of US childhood cancer survivors: relative risk of offspring of irradiated mothers (with respect to unirradiated survivors), ovarian dose	Referent: 1 0.01-0.99 Gy 0.87 (0.55, 1.38)[i] 1.00-2.49 Gy 0.80 (0.33, 1.92)[i] ≥2.50 Gy 0.59 (0.20, 1.75)[i] p-value trend 0.53
Stahl *et al*. [101]	Congenital abnormalities in offspring of male Danish and Swedish cancer survivors: relative risk of congenital abnormalities in offspring (with respect to those of fathers with no history of cancer)	Any major anomaly :1.17 (1.05, 1.31) Any anomaly: 1.12 (1.02, 1.24)
Garsi *et al*. [131]	Stillbirth in offspring of females given ^{131}I for thyroid cancer in terms of cumulative radioiodine activity	0 MBq 2.3% <370 MBq 2.0% 370-3700 MBq 0.0% >3700 MBq 0.6%
	Miscarriage in offspring of females given ^{131}I for thyroid cancer in terms of cumulative radioiodine activity	0 MBq 19.8% <370 MBq 18.4% 370-3700 MBq 15.3% >3700 MBq 21.4%
Goldberg *et al*. [126]	Unsuccessful attempt at pregnancy in offspring of females in relation to diagnostic dose for scoliosis in adolescence (in relation to general population reference)	Reference 1 0-0.00312 Gy 1.28 (0.6, 2.6) 0.00313-0.00689 Gy 1.10 (0.5, 2.2) 0.00690-0.01443 Gy 1.66 (0.9, 3.2) ≥0.01444 Gy 1.31 (0.7, 2.6)
	Spontaneous abortions in offspring of females in relation to diagnostic dose for scoliosis in adolescence (in relation to general population reference)	Reference 1 0-0.00312 Gy 1.76 (1.2, 2.4) 0.00313-0.00689 Gy 1.18 (0.8, 1.7) 0.00690-0.01443 Gy 1.09 (0.7, 1.7) ≥0.01444 Gy 1.32 (0.9, 1.9)
	Stillbirths in offspring of females in relation to diagnostic dose for scoliosis in adolescence (in relation to general population reference)	Reference 1 0-0.00312 Gy 0.26 (0.0, 2.0) 0.00313-0.00689 Gy 0.29 (0.0, 2.2) 0.00690-0.01443 Gy 0.35 (0.0, 2.7) ≥0.01444 Gy 0.52 (0.2, 1.8)
	Low birthweight (<2500 g) in offspring of females in relation to diagnostic dose for scoliosis in adolescence (in relation to general population reference)	Reference 1 0-0.00312 Gy 0.45 (0.2, 1.0) 0.00313-0.00689 Gy 0.64 (0.3, 1.2) 0.00690-0.01443 Gy 1.07 (0.6, 2.0) ≥0.01444 Gy 1.14 (0.7, 1.8)
	Low birthweight (<2500 g) in offspring of females in relation to diagnostic dose for scoliosis in adolescence (in relation to lowest quartile of scoliosis cohort)	0-0.00312 Gy 1 0.00313-0.00689 Gy 1.43 (0.5, 3.9) 0.00690-0.01443 Gy 2.24 (0.9, 5.9) ≥0.01444 Gy 2.34 (1.0, 5.6)
	Congenital malformations in offspring of females in relation to diagnostic dose for scoliosis in adolescence (in relation to general population reference)	Reference 1 0-0.00312 Gy 0.95 (0.4, 2.2)

		0.00313-0.00689 Gy 1.67 (0.9, 3.2) 0.00690-0.01443 Gy 1.64 (0.9, 3.1) ≥0.01444 Gy 1.24 (0.7, 2.3)
Signorello *et al*. [118]	Stillbirth and neonatal death in offspring of US childhood cancer survivors: fathers exposure (testicular irradiation)	0.8 (0.4, 1.6)[d]
	Stillbirth and neonatal death in offspring of US childhood cancer survivors: mothers exposure before menarche (dose to uterus or ovaries)	0.01-0.99 Gy 1.3 (0.5, 3.9)[d] 1.00-2.49 Gy 4.7 (1.2, 19.0)[d] ≥2.50 Gy 12.3 (4.2, 36.0)[d]
	Stillbirth and neonatal death in offspring of US childhood cancer survivors: mothers exposure after menarche (dose to uterus or ovaries)	0.01-0.99 Gy 0.3 (0.1, 1.0)[d] 1.00-2.49 Gy 1.2 (0.2, 6.4)[d] ≥2.50 Gy 0.2 (0.0, 1.4)[d]
Signorello *et al*.[125]	Preterm birth in offspring of female US childhood cancer survivors (relative risk, relative to offspring of female siblings)	Full-term birth (referent): 1 Pre-term birth: 1.9 (1.4, 2.4)[j]
	Preterm birth in offspring of female US childhood cancer survivors (relative risk, in relation to uterine dose pre-menarche)	0-0.09 Gy: 0.9 (0.5, 1.9)[b] 0.10-0.49 Gy: 2.2 (1.0, 4.8)[b] 0.50-2.49 Gy: 2.1 (1.0, 4.6)[b] >2.50 Gy: 4.9 (1.7, 13.9)[b]
	Preterm birth in offspring of female US childhood cancer survivors (relative risk, in relation to uterine dose post-menarche)	0-0.09 Gy: 1.2 (0.6, 2.4)[b] 0.10-0.49 Gy: 0.8 (0.3, 1.7)[b] 0.50-2.49 Gy: 1.8 (0.8, 4.3)[b] >2.50 Gy: 1.9 (0.7, 4.9)[b]
	Preterm birth in offspring of female US childhood cancer survivors (relative risk, in relation to ovarian dose)	0-0.09 Gy: 0.9 (0.6, 1.5)[b] 0.10-0.19 Gy: 1.2 (0.7, 2.4)[b] 0.20-0.49 Gy: 0.9 (0.4, 1.7)[b] 0.50-0.99 Gy: 1.5 (0.8, 3.0)[b] >1.00 Gy: 1.2 (0.4, 3.8)[b]
	Low birthweight in offspring of female US childhood cancer survivors (relative risk, relative to offspring of female siblings)	Non-low birth weight (referent): 1 Low birth weight: 1.3 (0.9, 1.9)[k]
	Small for gestational age (SGA) offspring of female US childhood cancer survivors (relative risk, relative to offspring of female siblings)	Non-SGA (referent): 1 SGA: 1.0 (0.8, 1.4)[l]
Winther *et al*. [119]	Spontaneous abortion in female Danish childhood cancer survivors, proportion ratios with sisters as referent	low/low dose 0.8 (0.3, 1.7)[h] low/high dose 1.8 (1.1, 3.0)[h] high/low dose 2.8 (1.7, 4.7)[h]
Winther *et al*. [102]	Sex ratio (male:female odds ratio) in offspring of males (irradiated vs unirradiated)	0.95 (0.74, 1.22)
	Sex ratio (male:female odds ratio) in offspring of males (irradiated vs unirradiated as function of dose group)	Unirradiated: 1 (referent) Low: 0.87 (0.55, 1.39) Low/medium: 0.79 (0.40, 1.56) Medium: 0.91 (0.55, 1.50) Medium/high: 0.96 (0.67, 1.38) High: 1.09 (0.65, 1.85) Unknown: 2.97 (0.31, 28.7)
	Sex ratio (male:female odds ratio) in offspring of females (irradiated vs unirradiated)	1.10 (0.85, 1.42)
	Sex ratio (male:female odds ratio) in offspring of females (irradiated vs unirradiated as function of dose group)	Unirradiated: 1 (referent) Low: 1.10 (0.77, 1.58) Low/medium: 1.08 (0.57, 2.06) Medium: 1.03 (0.47, 1.38)

		Medium/high: 1.09 (0.65, 1.85) High: 1.13 (0.61, 2.07)
Curwen *et al*. [68]	G2 mean chromosomal radiosensitivity difference between partners of Danish cancer survivors, cancer survivors and their offspring	112.00 (79 - 189)[m] (partners) 122.00 (73 - 160)[m] (survivors) 123.50 (73 - 404)[m] (offspring)
	Proportion of individuals with elevated G2 sensitivity (based on 90th percentile point from partner controls)	13% (partners) *vs* 4% (survivors) (p=0.608) *vs* 18% (offspring) (p=0.729)
	Proportion of individuals with elevated G2 sensitivity (based on 90th percentile point from Westlakes Research Institute (WRI) controls)	WRI 11% *vs* 35% (partners) (p=0.084) *vs* 52% (survivors) (p=0.002) *vs* 53% (offspring) (p<0.001)
Curwen *et al*. [69]	G2 chromosomal radiosensitivity difference between partners of Danish cancer survivors and their offspring	131.1±2.8m (partners) *vs* 137.2±2.9m (survivors) (p=0.576) *vs* 137.4±2.1m (offspring) (p=0.497)
	Proportion of individuals with elevated G2 sensitivity (based on 90th percentile point from partner controls)	3/29 (partners) *vs* 7/29 (survivors) (p=0.297) *vs* 11/53 (offspring) (p=0.358)
Tawn *et al*. [56]	Minisatellite mutations in offspring of Danish cancer survivors: fathers	0.97 (0.51, 1.68)[d]
	Minisatellite mutations in offspring of Danish cancer survivors: mothers	0.77 (0.29, 2.05)[d]
	Minisatellite mutations in offspring of Danish cancer survivors: fathers (in relation to mean testicular dose)	<0.10 Gy: 6.0%[n] 0.10-0.99 Gy: 6.5%[n] 1.00-1.99 Gy: 4.3%[n] ≥2.00 Gy: 5.5%[n]
Tawn *et al*. [39]	Genomic instability (chromatid aberrations and chromosome gaps) in offspring of Danish cancer survivors: difference between partners of cancer survivors and their offspring	2.69 ± 0.36 x 10-3 (offspring) *vs* 4.04 ± 0.60 x 10-3 (survivors) (p=0.04) *vs* 4.23 ± 0.60 x 10-3 (partners) (p=0.019) 2.53 ± 0.39 x 10-3 (gonadal dose >0.05 Gy) *vs* 3.24 ± 0.84 x 10-3 (gonadal dose ≤0.05 Gy) (*p*>0.05)
	Genomic instability (chromatid aberrations only) in offspring of Danish cancer survivors: difference between partners of cancer survivors and their offspring	2.59 ± 0.35 x 10-3 (offspring) *vs* 3.95 ± 0.60 x 10-3 (survivors) *vs* 3.89 ± 0.57 x 10-3 (partners) 2.40 ± 0.38 x 10-3 (gonadal dose >0.05 Gy) *vs* 3.24 ± 0.84 x 10-3 (gonadal dose ≤0.05 Gy)[e] (*p*>0.05)
Winther *et al*. [132]	Chromosomal abnormalities in offspring of Danish cancer survivors: Down syndrome	1.07 (0.16, 5.47)[d]
	Chromosomal abnormalities in offspring of Danish cancer survivors: Turner syndrome	1.32 (0.17, 7.96)[d]
	Chromosomal abnormalities in offspring of Danish cancer survivors: abnormal karyotype	0.21% (children of cancer survivors) vs 0.21% (children of siblings)[f]
Vorobtsova *et al*. [67]	Exchangable chromosome aberrations: 0 Gy *ex vivo*	0.12±0.07 (parents exposed) vs 0.02±0.02 (parents unexposed)[o]
	Exchangable chromosome aberrations: 1.5 Gy *ex vivo*	24.60±1.06 (parents exposed) vs 21.50±0.79 (parents unexposed)[o]
	Double fragment chromosome aberrations: 0 Gy *ex vivo*	0.90±0.19 (parents exposed) vs 0.90±0.15 (parents unexposed)[o]

	Double fragment chromosome aberrations: 1.5 Gy *ex vivo*	16.7±0.92 (parents exposed) vs 17.10±0.72 (parents unexposed)[o]
	Chromatid aberrations: 0 Gy *ex vivo*	1.70±0.16 (parents exposed) vs 0.80±0.14 (parents unexposed)[o]
	Chromatid aberrations: 1.5 Gy *ex vivo*	3.10±0.43 (parents exposed) vs 1.70±0.26 (parents unexposed)[o]

[a]excess relative risk Sv^{-1}

[b]relative risk (observed/expected)

[c]standardised incidence ratio

[d]relative risk (exposed/unexposed)

[e]frequencies of chromatid aberrations and chromosome gaps

[f]after exclusion of hereditary cases

[g]dose to ovary/uterus or testes below diaphragm was classified as medium to high (1-40 Gy to ovary/uterus, 0.2-25 Gy to testes), dose to ovary/uterus or testes above diaphragm was classified as low (0.01-1 Gy to ovary/uterus, <0.2 Gy to testes)

[h]A/B dose classified as to (A) ovary/uterus or (B) to pituitary. Dose to ovary/uterus below diaphragm was classified as medium to high (1-40 Gy), dose to ovary/uterus above diaphragm was classified as low (0.01-1 Gy). Dose to pituitary was classified as high for brain tumours and leukaemia with cranial irradiation (5-50 Gy), and low for tumours below the diaphragm (0.01-1 Gy).

[i]adjusted for calendar year of birth, paternal age (for male analyses), and for calendar year of birth, maternal age (for female analyses).

[j]pre-term birth = birth of less than 37 weeks gestation, full-term birth = birth of 37 weeks gestation or longer.

[k]low birthweight = birthweight of less than 2.5 kg.

[l]small for gestational age (SGA) = birthweight in bottom 10% for infants of same sex born in same gestational week.

[m]mean aberration frequency per 100 cells.

[n]median minisatellite mutation rates (+range).

[o]percentage aberrations ± SD in offspring of parents who had radiotherapy or chemotherapy *vs* controls.

Table 2 Pre-conception exposure risk coefficients in the Japanese atomic bomb survivor first-generation (F_1) offspring cohort. All reported risks use values from the original reports

Reference	Endpoint	Excess relative risk /Sv (+95% CI)
Yoshimoto *et al.* [107], Little [105]	Leukaemia incidence in relation to total paternal preconceptional gonadal dose, age < 20	<-0.166 (<-0.166, 0.979)
	Leukaemia incidence in relation to total maternal preconceptional gonadal dose, age < 20	1.185 (<-0.233, 4.874)
	Leukaemia incidence in relation to total conjoint (paternal+maternal) preconceptional gonadal dose, age < 20	0.071 (<-0.147, 1.882)
Yoshimoto *et al.* [107], Little [106]	Leukaemia incidence in relation to paternal preconceptional gonadal dose, 6 months before conception, age < 20	<0 (<0, 100)
	Leukaemia incidence in relation to total conjoint (paternal + maternal) preconceptional gonadal dose, 6 months before conception, age < 20	<0 (<0, 70)
Little *et al.* [108]	Leukaemia incidence (ICD9 204-208) in relation to total preconceptional gonadal dose, age < 20	Paternal: <-0.166 (<-0.166, 0.979) Maternal: 1.185 (<-0.233, 4.874) Conjoint: 0.071 (<-0.147, 1.882)
	Lymphoma incidence (ICD9 200-202) in relation to total preconceptional gonadal dose, age < 20	Paternal: 1.024 (<-0.166, 6.861) Maternal: <-0.233 (<-0.233, 1.729) Conjoint: 0.160 (<-0.147, 2.605)
	Non-Hodgkin's lymphoma incidence (ICD9 200, 202) in relation to total preconceptional gonadal dose, age < 20	Paternal: 1.332 (<-0.166, 8.707) Maternal: <-0.233 (<-0.233, 2.131) Conjoint: 0.282 (<-0.147, 3.421)
	Leukaemia and non-Hodgkin's lymphoma incidence (ICD9 200, 202) in relation to total preconceptional gonadal dose, age < 20	Paternal: 0.015 (<-0.166, 1.563) Maternal: 0.403 (<-0.233, 2.652) Conjoint: 0.166 (<-0.147, 1.492)
	Cancers other than leukaemia and non-Hodgkin's lymphoma incidence in relation to total preconceptional gonadal dose, age < 20	Paternal: <-0.166 (<-0.166, 0.929) Maternal: 0.510 (<-0.233, 2.802) Conjoint: -0.055 (<-0.147, 1.187)
	All cancer incidence in relation to total preconceptional gonadal dose, age < 20	Paternal: -0.164 (<-0.166, 0.677) Maternal: 0.457 (<-0.233, 1.890) Conjoint: 0.068 (<-0.147, 0.869)
	Mortality from diseases of the blood and blood-forming organs (ICD9 280-289), age < 40	Paternal: <-0.166 (<-0.166, 2.296) Maternal: <-0.233 (<-0.233, 2.543) Conjoint: <-0.147 (<-0.147, 1.167)
Yoshimoto *et al.* [127], Little [105]	Respiratory disease mortality, born 1/1951-12/1984, age < 40, paternal preconceptional dose	0.522 (0.091, 1.143)
	Digestive disease mortality, born 1/1951-12/1984, age < 40, paternal preconceptional dose	0.448 (-0.052, 1.253)
	All cause mortality, paternal preconceptional dose, born 1948-1958	0.009 (-0.089, 0.107)
Izumi *et al.* [109]	Solid cancer incidence in relation to parental dose	Paternal, age 1-19 0.3 (-1.6, 1.4) Paternal, age 20+ -0.4 (-0.8, 0.0) Maternal, age 1-19 0.2 (-1.2, 1.2) Maternal, age 20+ 0.1 (-0.2, 0.4)
	Haemopoietic cancer incidence in relation to parental dose	Paternal, age 1-19 -0.3 (-2.7, 0.9) Paternal, age 20+ -0.9 (-3.5, 0.7) Maternal, age 1-19 0.4 (-1.1, 1.3)

		Maternal, age 20+ -0.3 (-1.7, 0.7)
Izumi *et al.* [110]	Cancer mortality in relation to parental dose	Paternal, age 1-19 -9.6 (-10.0, 4.3) Paternal, age 20+ -3.6 (-7.1, 1.6) Maternal, age 1-19 3.8 (-5.6, 19.4) Maternal, age 20+ -0.8 (-4.3, 3.9)
	Non-cancer mortality in relation to parental dose	Paternal, age 1-19 1.6 (-1.0, 4.6) Paternal, age 20+ 0.1 (-3.3, 4.2) Maternal, age 1-19 -2.3 (-4.5, 0.3) Maternal, age 20+ 1.5 (-2.1, 5.9)
Fujiwara *et al.* [111]	Adult-onset multifactorial disease (diabetes mellitus, hypercholesterolaemia, hypertension, myocardial infarction, angina pectoris, stroke): regression coefficient (EOR/Sv) of paternal dose adjusted for maternal dose	-0.09 (-0.19, 0.01)
	Adult-onset multifactorial disease (diabetes mellitus, hypercholesterolaemia, hypertension, myocardial infarction, angina pectoris, stroke): regression coefficient (EOR/Sv) of maternal dose adjusted for paternal dose	-0.02 (-0.14, 0.10)
Tatsukawa *et al.* [112]	Hypertension (OR at 1 Sv)	Paternal dose 0.99 (0.86, 1.13) (p=0.84) Maternal dose 1.03 (0.89, 1.18) (p=0.73) Conjoint dose 1.01 (0.91, 1.11) (p=0.91)
	Hypercholesterolaemia (OR at 1 Sv)	Paternal dose 0.92 (0.83, 1.02) (p=0.12) Maternal dose 1.02 (0.91, 1.15) (p=0.71) Conjoint dose 0.96 (0.87, 1.05) (p=0.32)
	Diabetes mellitus (OR at 1 Sv)	Paternal dose 0.85 (0.67, 1.09) (p=0.21) Maternal dose 1.02 (0.81, 1.28) (p=0.87) Conjoint dose 0.91 (0.76, 1.09) (p=0.30)
	Angina pectoris (OR at 1 Sv)	Paternal dose 0.60 (0.27, 1.33) (p=0.21) Maternal dose 0.90 (0.51, 1.61) (p=0.74) Conjoint dose 0.77 (0.43, 1.37) (p=0.37)
	Myocardial infarction (OR at 1 Sv)	Paternal dose 0.56 (0.22, 1.40) (p=0.21) Maternal dose 0.54 (0.13, 2.22) (p=0.39) Conjoint dose 0.59 (0.22, 1.58) (p=0.29)
	Stroke (OR at 1 Sv)	Paternal dose 0.93 (0.57, 1.52) (p=0.77) Maternal dose 0.60 (0.25, 1.46) (p=0.26) Conjoint dose 0.83 (0.46, 1.48) (p=0.52)
	All endpoints combined (OR at 1 Sv)	Paternal dose 0.93 (0.86, 1.01) (p=0.07) Maternal dose 1.01 (0.93, 1.10) (p=0.76) Conjoint dose 0.96 (0.90, 1.03) (p=0.24)
Neel and Schull [116]	Congenital malformations in Hiroshima by approximate paternal dose group (1=least exposed, 5=most exposed)	1 (unexposed) 0.92% 2: 1.21% 3: 1.18% 4-5: 0.88%
	Congenital malformations in Hiroshima by approximate maternal dose group (1=least exposed, 5=most exposed)	1 (unexposed) 0.95% 2: 1.06% 3: 0.67% 4-5: 1.29%

	Congenital malformations in Nagasaki by approximate paternal dose group (1=least exposed, 5=most exposed)	1 (unexposed) 0.87% 2: 0.78% 3: 0.93% 4-5: 1.05%
	Congenital malformations in Nagasaki by approximate maternal dose group (1=least exposed, 5=most exposed)	1 (unexposed) 0.89% 2: 0.78% 3: 1.30% 4-5: 0.81%
Otake *et al.*[117]	Regression coefficient of absolute risk of stillbirth in relation to conjoint parental gonadal dose	0.00151 (SD 0.00199)
	Regression coefficient of absolute risk of neonatal death in relation to conjoint parental gonadal dose	0.00237 (SD 0.00233)
Yoshimoto *et al.* [127]	Birthweight chi-square heterogeneity analysis by parental gonadal dose group	$\chi^2_{15} = 27.07$, $P[\chi^2_{15} \geq 27.07] = 0.03$
Schull *et al.* [123]	Sex ratio	Paternal dose coefficient: -0.20 Gy^{-1} (NS) Maternal dose coefficient: -0.35 Gy^{-1} (NS)
Kodaira *et al.*[17]	*Pc-1* locus	0/65 (0%) in exposed *vs* 0/183 (0%) in controls
	λTM-18 locus	0/65 (0%) in exposed *vs* 0/183 (0%) in controls
	ChdTC-15 locus	0/65 (0%) in exposed *vs* 0/183 (0%) in controls
	pλg3 locus	1/65 (1.5%) in exposed *vs* 0/183 (0%) in controls
	λMS-1 locus	1/65 (1.5%) in exposed *vs* 11/183 (6.0%) in controls
	CEB-1 locus	4/65 (6.2%) in exposed *vs* 11/183 (6.0%) in controls
Satoh *et al.* [14]	Minisatellite mutation rate	1.5% in exposed gametes *vs* 2.0% in control gametes (*p*=0.37)
	Microsatellite mutation rate	0% in exposed gametes *vs* 0.5% in control gametes
Kodaira *et al.* [15]	Minisatellite mutation rate (paternal exposed *vs* control group)	4.6% exposed *vs* 4.7% unexposed: difference = -0.07% (-2.89, 3.36)
	Minisatellite mutation rate (maternal exposed *vs* control group)	0.8% exposed *vs* 0.9% unexposed: difference = -0.08% (-1.36, 1.62)
Kodaira *et al.* [16]	Microsatellite mutation rate (paternal exposed *vs* control group)	0.37% exposed *vs* 0.45% unexposed (*p*=0.73)
	Microsatellite mutation rate (maternal exposed *vs* control group)	0.26%/0.42% exposed *vs* 0.13%/0.17% unexposed (*p*=0.06/0.73)
Awa *et al.*[40]	All chromosome aberrations	0.517% exposed *vs* 0.639% controls
	Sex chromosome aberrations	0.228% exposed *vs* 0.301% controls
	Balanced autosomal rearrangements	0.216% exposed *vs* 0.313% controls
	Unbalanced autosomal rearrangements	0.060% exposed *vs* 0.025% controls
	Autosomal trisomy	0.012% exposed *vs* 0% controls

Table 3 Pre-conception exposure risk coefficients in various occupationally-and environmentally-exposed groups. All reported risks use values from the original reports

Reference	Endpoint	Risk (and 95% CI)
Draper *et al*. [8]	Leukaemia and non-Hodgkin's lymphoma incidence in offspring of UK radiation workers in relation to total paternal preconceptional gonadal dose, age < 15	5.2 (-2.9, 27.6)[a]
	Leukaemia and non-Hodgkin's lymphoma incidence in offspring of UK radiation workers in relation to 6-month paternal preconceptional gonadal dose, age < 15	34 (-33, 218)[a b]
	Leukaemia and non-Hodgkin's lymphoma incidence in offspring of UK radiation workers in relation to 3-month paternal preconceptional gonadal dose, age < 15	10 (-106, 290)[a b]
	All cancer incidence in offspring of UK radiation workers in relation to total paternal preconceptional gonadal dose, age < 15	11.3 (NA, 37.9)[a]
	All cancer incidence in offspring of UK radiation workers in relation to 6-month paternal preconceptional gonadal dose, age < 15	42 (-20, 181)[a b]
	All cancer incidence in offspring of UK radiation workers in relation to 3-month paternal preconceptional gonadal dose, age < 15	38 (-68, 200)[a b]
Bunch *et al*.[133]	Leukaemia and non-Hodgkin's lymphoma incidence in offspring of female UK radiation workers, no in utero employment, age < 15	1.00 (0.19, 5.37)[c]
	All cancers apart from leukaemia and non-Hodgkin's lymphoma incidence in offspring of female UK radiation workers, no in utero employment, age < 15	1.60 (0.46, 6.22)[c]
Roman *et al*. [113]	All cancer incidence in offspring of UK male radiation workers, age < 25: cumulative preconception dose	<50 mSv: 1.4 (0.8, 2.2)[e] 50-99 mSv: 1.3 (0.5, 3.8)[e] ≥100 mSv: 2.2 (0.9, 5.3)[e]
	All cancer incidence in offspring of UK male radiation workers, age < 25: 6-month preconception dose	<5 mSv: 1.3 (0.8, 2.2)[e] 5-9.9 mSv: 1.4 (0.4, 4.5)[e] ≥10 mSv: 2.5 (1.0, 6.5)[e]
	Leukaemia and NHL incidence in offspring of UK male radiation workers, age < 25: cumulative preconception dose	<50 mSv: 1.7 (0.6, 4.3)[e] 50-99 mSv: 1.2 (0.2, 9.4)[e] ≥100 mSv: 3.9 (1.0, 15.7)[e]
	Leukaemia and NHL incidence in offspring of UK male radiation workers, age < 25: 6-month preconception dose	<5 mSv: 1.7 (0.6, 4.3)[e] 5-9.9 mSv: 0.0 (NA, NA)[e] ≥10 mSv: 5.4 (1.4, 20.5)[e]
McLaughlin *et al*.[134]	Leukaemia incidence in offspring of Ontario radiation workers in relation to total paternal preconceptional gonadal dose, age < 15	0.87 (0.32, 2.34)[d]
Parker *et al*. [120]	Stillbirth in offspring of Sellafield radiation workers in relation to total paternal preconceptional gonadal dose	2.4 (0.4, 4.5)[a]
Mudie *et al*. [135]	Sex ratio (male:female odds ratio) in offspring of mothers exposed via nuclear weapons test in Kazakhstan	<0.20 Gy: 1 0.20-0.399 Gy: 1.01 (0.92, 1.11) 0.40-0.599 Gy: 1.04 (0.94, 1.15) ≥0.60 Gy: 1.08 (0.97, 1.20) trend *p*=0.42
Maconochie *et al*. [124]	Sex ratio (male:female odds ratio) in offspring of UK nuclear workers	0 Sv: 1 >0-0.0024 Sv: 1.03 (0.42, 2.55) 0.0025-0.0099 Sv: 0.94 (0.38, 2.29) 0.010-0.0199 Sv: 1.30 (0.51, 3.30)

		0.020-0.0499 Sv: 0.73 (0.29, 1.81) ≥0.050 Sv: 1.35 (0.38, 4.76) heterogeneity *p*=0.46
Dubrova *et al*. [10]	Minisatellite mutation rate in offspring of Chernobyl-exposed Belarus families and UK controls	0.0303 (exposed) vs 0.0154 (control) *p*=0.0041
Dubrova *et al*. [11]	Minisatellite mutation rate in offspring of Chernobyl-exposed Belarus families and UK controls	0.0206 (exposed) vs 0.0110 (control) *p*=2.53 x 10-5
Dubrova *et al*. [12]	Minisatellite mutation rate in (offspring of) families in exposed (to > 1 Sv) Beskaragai region of Kazakhstan and control families in Taldy Kurgan region	1.5f *p*=0.0476
Dubrova *et al*. [136]	Minisatellite mutation rate in offspring of Chernobyl-exposed Ukraine families: paternal exposure	6.41% (exposed) vs 4.11 (control)(*p*=0.0299)
	Minisatellite mutation rate in offspring of Chernobyl-exposed Ukraine families: maternal exposure	1.45% (exposed) vs 1.43 (control)(*p*=1.00)
Dubrova *et al*. [58]	Minisatellite mutation rate in offspring of Techa-river-exposed families: paternal exposure	4.88% (exposed) vs 2.91 (control) (*p*=0.0341)
	Minisatellite mutation rate in offspring of Techa-river-exposed families: maternal exposure	0.61% (exposed) vs 1.13 (control) (*p*=0.3775)
Livshits *et al*. [21]	Minisatellite mutation rate per band in offspring of Chernobyl-exposed Ukraine male liquidators	CEB1 band: 0.15 (exposed) vs 0.14 (control) (*p*=0.76) CEB15 band: 0.01 (exposed) vs 0.03 (control) (*p*=0.26) CEB25 band: 0.12 (exposed) vs 0.07 (control) (*p*=0.12) CEB36 band: 0.02 (exposed) vs 0 (control) (*p*=0.13) CEB42 band: 0.01 (exposed) vs 0.01 (control) (*p*=1.00) CEB72 band: 0.01 (exposed) vs 0.03 (control) (*p*=0.44) B6.7 band: 0.08 (exposed) vs 0.08 (control) (*p*=1.00)
	Comparisons of minisatellite mutation rate among offspring conceived during or shortly after (subgroup 1) vs offspring conceived > 4 months after Chernobyl (subgroup 2), among Ukraine male liquidators (rate ratio (RR) subgroup 1: subgroup 2)	CEB1 band: RR 1.44 (*p*=0.31) CEB15 band: RR - (*p*=0.50) CEB25 band: RR - (*p*=0.26) CEB36 band: RR 0.35 (*p*=0.62) CEB42 band: RR - (*p*=0.25) CEB72 band: RR - (*p*=0.24) B6.7 band: RR 0.80 (*p*=0.77) Total: RR 1.12 (*p*=0.64)
Furitsu *et al*. [20]	Microsatellite mutation rate in offspring of Chernobyl-exposed Belarussian male liquidators	Y-linked loci: 2.9 x 10-3 (exposed) vs 2.1 x 10^{-3} (control) (*p*=0.950) Autosomal loci: 5.9 x 10^{-3} (exposed) vs 8.5 x 10^{-3} (control) (*p*=0.339)
Slebos *et al*.[19]	Minisatellite mutation rate in offspring of Chernobyl-exposed Ukraine liquidators (children conceived after vs before accident)	1.01% (exposed) vs 1.50 (control) (*p*>0.20)
	Microsatellite mutation rate in offspring of Chernobyl-exposed Ukraine liquidators (children conceived after vs before accident)	D3S1531 marker: 0/67 (exposed) vs 0/38 (control) (*p*>0.20) D7S1482 marker: 3/67 (exposed) vs 1/35 (control) (*p*>0.20) D20S82 marker: 0/72 (exposed) vs 0/38 (control) (*p*>0.20) D21S1245 marker: 2/47 (exposed) vs 1/26 (control) (*p*>0.20) DXS981 marker: 1/72 (exposed) vs 0/37 (control) (*p*>0.20)

Kiuru *et al*. [18]	Minisatellite mutation rate in offspring of male Chernobyl-exposed Estonian liquidators (odds ratio of children conceived after vs before accident)	1.33 (0.80, 2.20)
	Minisatellite mutation rate in offspring of male Chernobyl-exposed Estonian liquidators (odds ratio of children conceived after vs before accident in relation to paternal dose)	0.043-0.099 Sv 0.95 (0.44, 2.05) 0.100-0.199 Sv 1.14 (0.47, 2.77) 0.200-0.300 Sv 3.00 (0.97, 9.30)
Weinberg et al. [32]	New DNA mutations in offspring of Chernobyl liquidators (children conceived before or after Chernobyl exposure)	$p<0.002$ (Israel) $p<10\text{-}6$ (Ukraine)

[a]excess relative risk Sv^{-1}

[b]using exponential relative risk model

[c]relative risk (relative to non-radiation workers)

[d]relative risk (of persons with ≥0.1 mSv preconception vs 0 mSv preconception)

[e]relative risk, adjusted for calendar period, age and sex of child, and number of children born to each parent

[f]relative risk in offspring of exposed *vs* controls